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Oxidized LDL Damages Endothelial Cell Monolayer and Promotes Thrombocyte Adhesion

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The influence of oxidized low density lipoprotein (LDL) on a human endothelial cell monolayer was examined. The resulting contraction of the oxidized LDL-damaged endothelial cells lets intercellular spaces become enlarged and therefore visible via light microscopy. Electron microscopy reveals that the structural damage facilitates thrombocyte adhesion and formation of microthrombi. Oxidized LDL appears to play a pivotal role in initiating and deteriorating thromboembolic complications. Am. J. Hematol. 57: 341–343, 1998. © 1998 Wiley-Liss, Inc.

Key words: oxidized LDL; endothelial cell monolayer; cytodamage; atherogenesis

INTRODUCTION

It is generally accepted that the integrity of the lining vascular endothelium as part of Virchow's triad serves as a major inhibitor of coagulation and thrombus formation. Destroying the protective properties of endothelial cells is shown to have a devastating effect on coronal atherosclerotic patients.

In vivo, a certain percentage of circulating LDL is found in an oxidized state. Certain cells as well as transitional metal ions, such as iron and copper, are held responsible for these oxidative changes in circulating and parietal LDL [1]. The cells capable of oxidizing LDL comprise smooth muscle cells, monocytes, macrophages, but also endothelial cells [2–4]. Atherosclerotic walls of arteries are thought to contain an especially high amount of the transitional metal ions and, therefore, may amplify the devastating process of atherosclerosis [1].

Several studies have already documented that oxidized LDL stimulates thrombocytes and causes serotonin release [5]. It is the aim of this work to observe whether oxidized LDL promotes the formation of microthrombi on cultured human endothelial cell monolayer.

MATERIALS AND METHODS

The preparations of oxidized LDL and washed human thrombocytes were carried out as previously outlined [6].

The human umbilical vein endothelial cells (HUVEC) used in this study were purchased from Clonetics Corporation (San Diego, CA) and utilized in the present experiments at passage 4. Endothelial cell confluence was usually achieved within 2–3 days after plating and the monolayers were utilized for experiments within 5 days. Prior to each experiment, the cells were examined for any abnormalities that might indicate non-confluent or atypical HUVEC monolayers.

In culture flasks, oxidized LDL and a concentration of 200 μg protein/ml in serum-free DMEM was used to expose endothelial cells. Native LDL, at the same concentration, was added to the serum-free DMEM in the control group. The procedure lasted for 12 hr and was conducted in the CO_2-incubator at 37°C. The washed thrombocytes were adjusted to a concentration of 2×10^3 cells/ml with a medium containing 1 mM $CaCl_2$ and added into the culture flasks, which were then put into the incubator for a further 30 min. The monolayers were examined under light and scanning electron microscopy.

RESULTS

Light Microscopic Evaluation

Endothelial cells exposed to native LDL showed no morphological change in comparison to cells used as the control. Figure 1A shows normal spindle-shaped endothelial cells of average size. Most of the cells of the cultured endothelial cell monolayer markedly demon-

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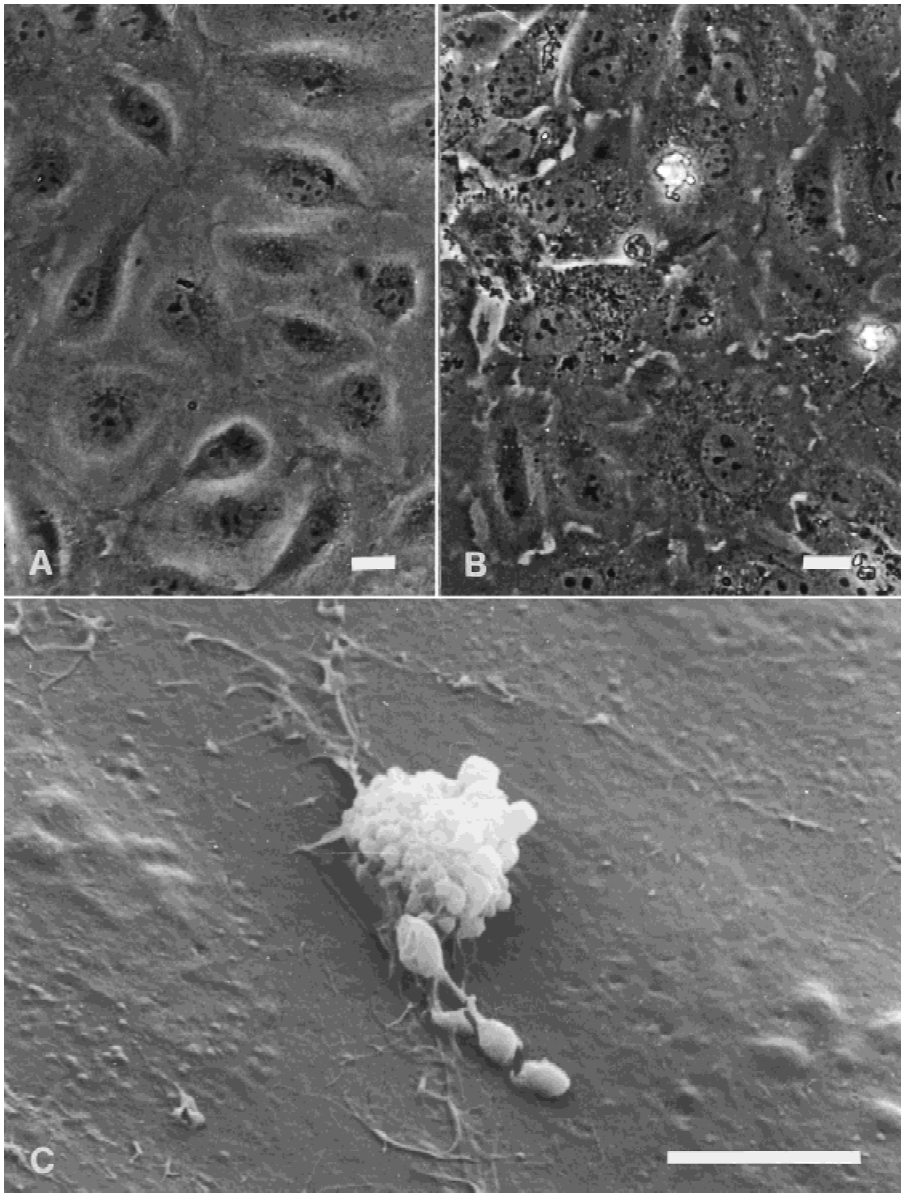

Fig. 1. Light (A, B) and scanning electron (C) micrographs of human endothelial cell monolayer. A: Control. B, C: Treated by oxidized LDL. B reveals increased intercellular gaps. C shows a microthrombus adhered to the damaged surface of the endothelial monolayer. Bars: 10 µm.

strate cell-to-cell contact. The intracellular spaces, therefore, are barely visible. However, a comparison of oxidized LDL-treated and untreated endothelial cells showed significant differences with regard to changes in morphology. Figure 1B reveals smaller, deformed cells demonstrating areas with little or no cell-to-cell contact between adjacent cells. Intercellular gaps have appeared, most likely as a result of the contraction of the injured endothelial cells due to exposure to oxidized LDL.

Scanning Electron Microscopy

Scanning electron microscopic evaluation illustrates the attachment of thrombocytes and fibrinous material to the endothelial walls due to previously occurring structural damage to the endothelial cell monolayer. Some microthrombi adhere to the damaged surface of the endothelial monolayers (Fig. 1C). These phenomena are observed only in the oxidized LDL-treated groups, but not in the native LDL groups and control groups.

DISCUSSION

It is reported that a certain percentage of circulating LDL undergoes a constant conversion from the reduced state into the oxidized state. Especially unsaturated fatty acids from triglycerides and phospholipids are subject to

oxidation as well as amino acids from apolipoproteins that are contents of LDL. Among other cells that are responsible for oxidation of LDL, such as monocytes, macrophages, and smooth muscle cells, even endothelial cells stimulate the oxidative change of LDL and, therefore, contribute indirectly to their own destruction [1]. Furthermore, in atherosclerotically altered arterial walls an increased number of transitional metal ions, such as copper and iron, can often be encountered. These transitional metal ion deposits also induce the oxidative alteration of LDL and, therefore, may play a significant role in accelerating an already existent process of atherosclerosis [1].

Recently, we reported an oxidized LDL-induced increase in the amount of intracellular calcium leading to changes in cytoskeletal f-actin distribution [7]. Our previous data show that even a very small amount of oxidized LDL is able to increase the intracellular calcium concentration with long-term exposure [7]. This chronic increase of intracellular calcium ions may act via intermediary release of vasoactive factors from endothelial cells on the modification of cytoskeletal protein and f-actin distribution, thereby reorganizing the cytoskeleton [7,8]. Hence, the possible consequences of a deranged cytoskeleton should be strongly taken into consideration. The altered cytoskeleton could have turned the spindle shape into a rounder appearance as creating the markedly noticeable gaps between the adjacent endothelial cells (Fig. 1B). This change is visible under light microscopy in the present study. Since the structural damage facilitates thrombocyte adhesion and formation of microthrombi, oxidized LDL appears to play a pivotal role in initiating and deteriorating thromboembolic complications.

Endothelial cells are intimately involved with a variety of biochemical and physiological reactions. Although the mechanisms by which the oxidized LDL affects endothelial cells in vivo are not entirely clear, we share the opinion that prophylaxis of this long-term process may be achieved by antioxidative vitamins and other antioxidants [9–12]. Early onset of these preventative methods may help to substantially slow the process of developing atherosclerosis and coronary heart disease, especially in high-risk patients with a family history of a coagulative disease.

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